The effects of GnRH analogs on serum and follicular fluid leptin levels and pregnancy outcomes in short protocols of assisted reproductive technology

Yardımcı üreme tekniklerinde kısa protokol uygulamalarında GnRH analoglarının serum ve foliküler leptin seviyeleri ve gebelik sonuçları üzerine etkileri

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Abstract

Objective: To determine serum and follicular leptin levels in patients using gonadotropin releasing hormone agonist and antagonist in Assisted Reproductive Technology short protocol cycles and to evaluate pregnancy outcomes.

Material and Methods: Patients randomly selected to join assisted reproductive technology cycles during February 2004-July 2004 were enrolled in this study. Group 1 consisted of 21 patients receiving r FSH+ GnRH agonists, whereas Group 2 consisted of 34 patients who received r FSH +GnRH antagonists. During the ovulation induction period 5 serum samples were collected (induction day 1, day 3 or antagonist starting day, human chorionic hormone day, oocyte pickup day, and twelfth day of embryo transfer). Follicular fluid samples were collected to be evaluated for leptin, estradiol, prolactin and luteinizing hormone.

Results: There was no difference in age, basal FSH, basal LH, and basal E2 between groups. Serum leptin levels were similar in both groups. Also, when each group's serum leptin levels were evaluated according to the presence of pregnancy, there was no significant difference in both groups. When follicle leptin levels were evaluated according to the existence of pregnancy, in both groups the follicle leptin levels were lower in pregnant participants but this difference was not statistically significant. When obesity is defined as body mass index over 26.5, there is a correlation between obesity and leptin levels in Group 2.

Conclusion: Our results have shown that both agonists and antagonists have similar efficacy and effect in poor responder women. Leptin levels in either groups, whether pregnant or non-pregnant were not statistically different. This result shows the need for more studies on leptin in infertility.

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Key words: GnRH agonist, GnRH antagonist, ART, leptin, prolactin

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Özet

Amaç: Yardımcı Üreme Teknikleri (YÜT) ile kısa protokol uygulanan sikluslarda gonadotropin salgılatıcı hormon (GnRH) agonist ve antagonist uygulanan olgularda serum ve foliküler leptin seviyelerinin belirlenmesi ve gebelik sonuçlarının değerlendirilmesidir.

Gereç ve Yöntemler: Çalışmaya dahil edilen hastalar random olarak Şubat 2004-Temmuz 2004 tarihleri arasında yardımcı üreme teknik siklusları uygulanan olgular arasından seçildi. Grup 1 rekombinant FSH+ GnRH agonist uygulanan 21 hastayı içerirken Grup 2 rekombinant FSH +GnRH antagonist uygulanan 34 hastayı içermekte idi. Ovulasyon indüksiyonu sürecinde 5 serum örneği alındı (indüksiyonun 1. günü, 3. günü veya antagonist başlandığı gün, insan koryonik hormon uygulama günü, oosit aspirasyon günü ve embryo transferi sonrası 12. gün). Foliküler sıvı örnekleri leptin, östradiol, prolaktin and luteinize edici hormon değerlendirilmesi amacı ile alındı.

Bulgular: Gruplar arasında yaş, bazal FSH, bazal LH ve bazal E2 açısından fark izlenmedi. Serum leptin düzeyleri her iki grupta benzer idi. Ayrıca her grupta gebelik varlığına göre serum leptin seviyeleri değerlendirildiğinde gruplar arasında fark izlenmedi. Folikül leptin seviyeleri gebelik mevcudiyetine göre değerlendirildiğinde her iki grupta da folikül leptin seviyeleri gebe olgularda daha düşük olmasına rağmen bu fark istatistiksel olarak anlamlı değildi. Obesite vücut kitle indeks değerinin 26.5 üzerinde olması olarak tanımlandığında Grup 2'de leptin seviyeleri ile obesite arasında korelasyon mevcut idi.

Sonuç: Sonuçlarımız düşük cevaplı olgularda hem agonistlerin hem de antagonistlerin benzer etkinlik ve etkiye sahip olduğunu göstermiştir. Her iki grupta da leptin seviyeleri gebe olanlarda veya olmayanlarda istatistiksel olarak farklı izlenmedi. Bu sonuçlar leptinin infetilitedeki yeri ile ilgili daha fazla çalışmaya ihtiyaç olduğunu göstermektedir.

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Anahtar kelimeler: GnRH agonisti, GnRH antagonisti, YÜT, leptin, prolaktin

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Introduction

Leptin, a 167-amino-acid product of the human leptin gene, is primarily expressed in adipose tissue, but it is also found in many other tissues, including the placenta, mammary gland, testes, ovary, endometrium, stomach, hypothalamus, pituitary, and others (1). It was first found through positional cloning of ob./ob. mice at the Jackson Laboratories (2). These mice had a homozygous mutation of the leptin gene. This mutation results in clinical hyperphagia, extreme obesity, dia-

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betes, neuroendocrine abnormalities, and infertility because of the incomplete leptin deficiency.

Leptin mainly plays a role in the regulation of body weight and energy homeostasis by signaling the amount of energy stores. In addition, leptin also plays a key role in human reproduction especially the hypothalamic leptin receptors that modulate the secretion of gonadotropin-releasing hormone (GnRH). Moreover some researchers suggested that leptin also has a direct role in the ovary and endometrium as well as the central actions. Leptin and its receptors were also shown to be synthesized by the granulosa cells in the ovary. The same leptin may inhibit FSH induced estradiol synthesis in granulosa cells (3-5).

The effects of leptin are mediated by receptors which are encoded by the LEPR gene found in 1q31 chromosome and belong to the gp130 family of receptors. Five leptin receptors have been described (Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, Ob-Re). Ob-Rb is considered to be the main functional receptor expressed in the hypothalamus and in other sites as well (6). Leptin is secreted in a pulsatile fashion and has a significant diurnal variation, with higher levels in the evening and early morning hours. With higher levels during the luteal phase, it shows a considerable variation throughout the human menstrual cycle (7, 8).

Overall, leptin may have both direct and indirect effects on follicular growth and oocyte development as well as implantation. Although serum or follicular fluid (FF) leptin levels and its effects on pregnancy outcome has been studied by many authors, there are few studies that have compared pregnancy results of both agonist and antagonist treatment protocols through leptin levels. In this study we aimed to investigate the association between both serum and follicular fluid leptin levels and pregnancy outcomes in patients who were treated by a short protocol with GnRH agonist and antagonist in ARTcycles.

Methods

The study was carried out in the University Family Planning and Infertility Research and Treatment Center. The study protocol was approved by the Local Ethics Committee. Fifty-five women aged 20-40 years were enrolled in the study after giving their informed consent. Duration of infertility ranged between 6 months to 20 years. Inclusion criteria of infertility were male factor, tubal factor and idiopathic factor. Exclusion criteria were diagnosis of polycystic ovarian syndrome, any other medical illness and patients' desire to resign Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. For all patients, transvaginal sonography was performed in order to exclude ovarian and uterine pathologies.

Ovulation induction protocols

Group 1 (n=21) consisted of patients who were treated by GnRH agonists and gonadotropins as a short protocol. Ovarian stimulation was initiated on the 2nd menstrual day by administration of recombinant FSH (Gonal F, Serono International SA, Geneva, Switzerland- Puregon, Organon International, U.S.A.) and 0.1 mg GnRH analog (Decapeptyl, Ferring GmbhH Kiel, Germany). The dosage of recombinant FSH was adjusted according to the patients' individual requirements (age, basal FSH) and serum estradiol levels at day 3 of induction. Ovarian stimulation was monitored by sequential transvaginal ultrasonography and serum estradiol measurements in order to assess follicular development. Finally, human chorionic gonadotropin (hCG) 10.000 IU (Profasi HP 10000 Serono International S.p.A. Italy) was given subcutaneously when a consistent rise in serum estradiol concentrations was associated with the presence of two or more follicles of more than 18 mm diameter. Oocyte aspiration was performed by transvaginal ultrasonography 35-36 h after hCG injection.

Group 2 (n=34) consisted of participants who were treated with GnRH antagonist and gonadotropins. In group 2, ovulation inductions began on the second menstrual day by recombinant FSH (Gonal F, Serono International SA, Geneva, Switzerland-Puregon, Organon International, U.S.A.). The dosage of recombinant FSH was adjusted according to the patients' individual requirements (age, basal FSH) and serum estradiol levels on day 3 of induction. Ovarian stimulation was monitored by sequential transvaginal ultrasonography and when a 14 mm sized follicle was detected, daily administration of 0.25 μg GnRH antagonist (Cetrotide, Serano International SA, Geneva, Switzerland) was added to the treatment until the subcutaneous hCG injection. Criteria of hCG injection were the same as in Group 1.

Fertilization was achieved by intracytoplasmic sperm injection (ICSI) in both groups. Fertilization checks were performed 16-20 hours after ICSI. High quality embryos were transferred on the second or third day of ICSI after endometrial thickness measurements. For luteal phase support, 800 mg micronized transvaginal progesterone (Crinone gel, Serono International SA,Geneva, Switzerland) was administered once a day. Additionally, 2000 IU HCG was injected on transfer +1,+ 4, +7, and + 9. days.

Ovarian hyperstimulation was defined as the presence of simultaneous multifollicular development and estradiol levels over 2500 pg/ml.

Diagnosis of pregnancy was confirmed on embryo transfer (ET)+14 days, with hCG levels over 50 IU/l.

In both groups whole gonadotropin doses, duration of induction, number of aspirated oocytes, MI, PI, MII, fertilized oocytes and transferred embryos were recorded.

Serum Samples

Throughout the induction period, 5 separate blood and one FF sample were withdrawn in each group in the morning hours. In group 1 samples were taken: 1- first day of induction (FSH, LH, E2, prolactin, leptin) 2- third day of induction (E2, prolactin, leptin) 3- day of hCG administration (E2, LH, prolactin, leptin) 4- day of oocyte aspiration [serum (E2, prolactin, leptin), and FF (prolactin, leptin)] 5-12th day of embryo transfer (E2, HCG, prolactin, progesterone, leptin). In group 2, samples were taken as group 1 apart from the second sample. The second sample was withdrawn at the beginning of antagonist administration. All blood samples were centrifuged at 2000 xg for 10 minutes at room temperature and thesupernatant was stored at -80°C until examination. Analyses of estradiol, FSH, LH, prolactin, progester-

one and HCG were performed by ACS: 180® (Bayer HealthCare) automated chemiluminescence systems. Leptin was examined by immunoenzymometric assays (Biosource Europe S.A., Ninelles Belgium) in both serum and FF. Sensivity for leptin was 0.1 ng/ml and intraassay CV was 3.6% of 14.8 ng/ml.

Statistical analyses

Statistical package for social sciences (SPSS) 10.0 was used for evaluation of the statistical analysis. Data was presented as means±SD. ANOVA was used for continuous variables and Bonferroni as a post-hoc test where applicable. If data was not distributed normally the Mann Whitney U test was used. The Chi-square test was used for classified variables and Fisher exact test where needed. $p<0.05$ was considered as significant.

Results

There was no significant difference between group 1 (n=21) and group 2 (n=34) for age, weight, length, BMI, length of infertility, gonadotropin dosage, length of induction period, number of fertilized oocyte, transferred embryo and endometrial thickness (Table 1). Etiological classification of both groups was similar (Table 2). The number of aspirated oocytes was significantly greater in group 1 ($p<0.02$), but the pregnancy rate of each group was similar. When pregnants were compared with nonpregnants in both groups, the number of transferred embryos was significantly higher in pregnants (Table 3).

Basal hormonal parameters had similar values in both groups but in the hCG injection period LH values were significantly lower in group 2. Similar serum leptin levels were detected in both groups. When each group's serum leptin levels were evaluated according to the presence of pregnancy, there was also no significant difference in both groups (Figure 1a, b). In each group, leptin levels were different for each sample. In the period of hCG injection, levels of leptin in FF were significantly higher than serum in both groups. When FF leptin levels were evaluated according to the existence of pregnancy, in both groups FF leptin levels were lower in pregnant participants, but this difference was not statistically significant. Serum leptin levels also did not show correlation between serum estradiol and prolactin levels in both groups. Howev, when obesity was defined as BMI over 26.5, there was a correlation between obesity and leptin levels in Group 2.

Estradiol levels were significantly lower in the period of hCG injection and oocyte aspiration in group 2 compared to group 1. When estradiol levels were compared among pregnant women,

Table 1. Demographic data and treatment details of groups are listed

	Group	Mean values	Standart deviation	p value
Age	1	32.9	3,6	0.158
	2	34.3	3,1	
Weight (kg)	1	62.8	9,9	0.315
	2	66.2	11,5	
Height (cm)	1	161.4	5,7	0.992
	2	161.4	5,1	
BMI	1	24.0	3,5	0.382
	2	25.2	4,3	
Infertility duration	1	7.3	5,3	0.646
	2	8.2	5.6	
Gonadotrophin dose (U)	1	3178.5	956.6	0.573
	2	3327.9	928.6	
Gonadotrophin duration	1	8.7	1.4	0.056
	2	8.0	0.9	
Aspirated oocyte	1	11.2	6.5	0.020*
	2	7.4	5.7	
Fertilization	1	4.9	2.8	0.259
	2	4.3	3.4	
Transferred embryo	1	2.6	0.7	0.278
	2	2.3	0.9	
Endometrial thickness	1	11.1	2.3	0.391
	2	10.8	1.8	

BMI: Body mass index, *: $p<0.05$ (Mann Whitney U-test)

there was a significant difference and levels began to rise after oocyte aspiration in the pregnant participants in both groups (Figure 2a, b).

Prolactin levels in FF were detected significantly lower in group 2 than group 1 (28.9 ng/ml versus 45.5 ng/ml, $p<0.05$). When prolactin levels were evaluated through pregnancy, pregnant participants had lower levels than non pregnants in both groups, but this difference was not significant (Table 4).

When each group was evaluated for pregnancy outcomes there was no significant difference between the groups (Table 1).

Table 2. Distribution of infertility etiologies between groups

Etiology	Group 1 (n=21)		Group 2 (n=34)	
	%	n	%	n
Male factor	52.4	11	58.8	20
Tubal factor	33.3	7	20.6	7
Undefined	14.3	3	20.6	7

Table 3. Pregnancy number and rate of each group

	Pregnant	Non pregnant	Total
Group 1	7 (33%)	14 (66%)	21 (38.1%)
Group 2	17 (50%)	17 (50%)	34 (61.9%)
Total	24 (43.6%)	31 (56.4%)	55 (100%)

Discussion

In our study, we evaluated the effects of GnRH analogs to serum and FF leptin levels and pregnancy outcomes in short protocols of ART. The aim of treatment with GnRH analogs is mainly to prevent early LH increase. At the same time, it has beneficial effects in follicle synchronization (9, 10). Antagonists are generally part of short protocols, whereas agonists are generally combined with long protocols of controlled ovarian stimulation. When antagonists with short protocols are compared to an agonist with long protocols, antagonists express the advantage of low dose gonadotropin use, more precise and simple stimulation and lower incidence of hyperstimulation (11). Agonist supplementation to the short protocols provides ovarian stimulation in low responder patients without suppression effects,

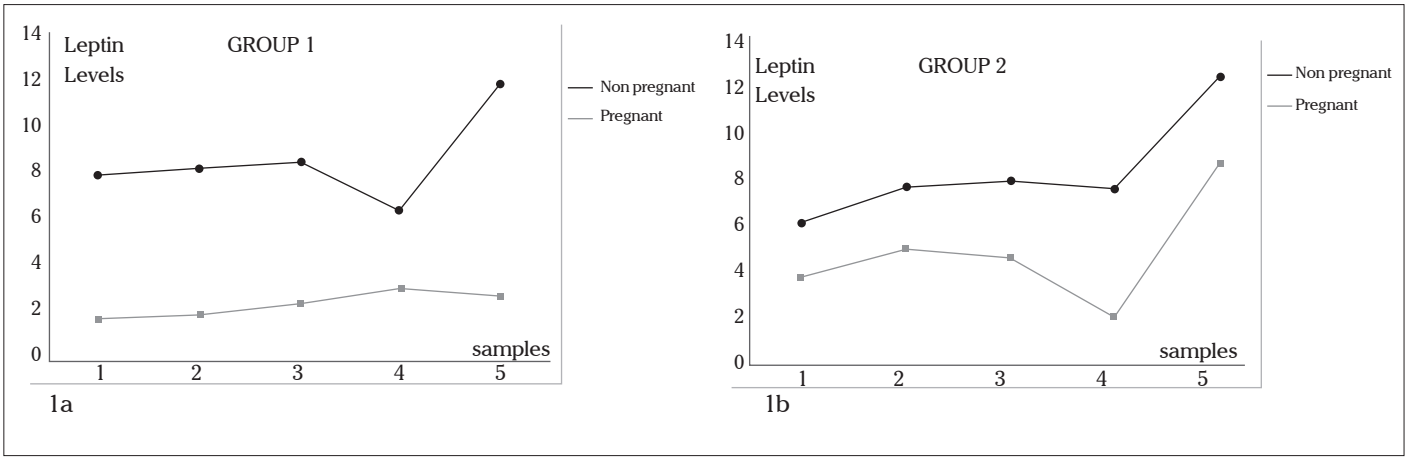

Figure 1a, b. When serum leptin levels of each group were evaluated according to the presence of pregnancy, there was no significant difference in both groups

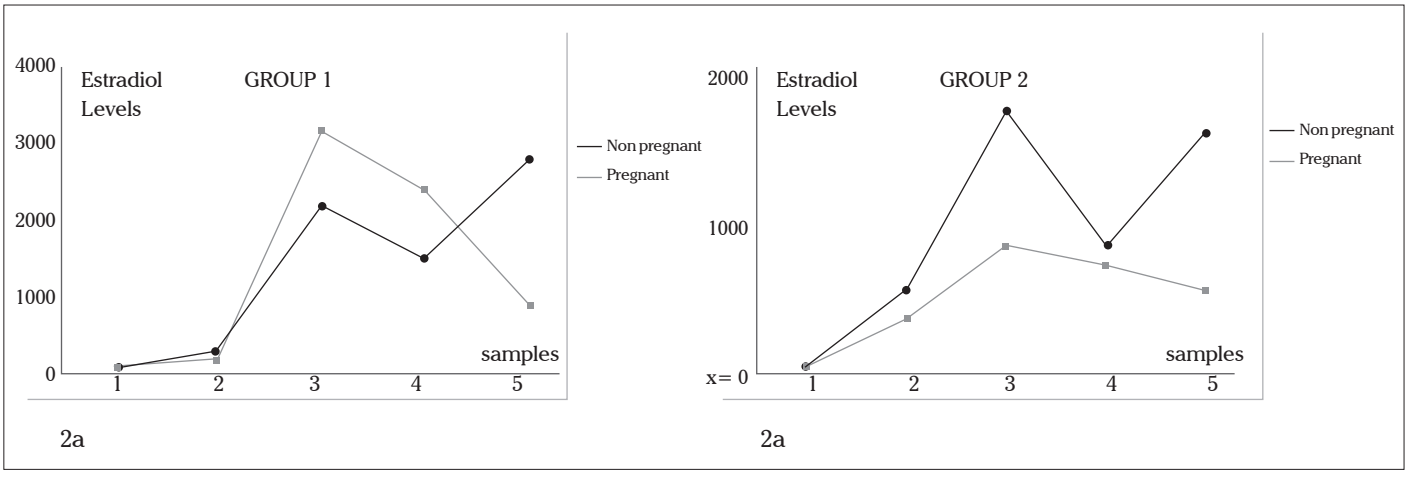

Figure 2a, b. When estradiol levels were compared among pregnant women, there was a significant difference and levels began to rise after oocyte aspiration in the pregnant participants in both groups

Table 4. The relationship of pregnancy and number of transferred embryo and FF prolactin levels

	Pregnancy	Mean number of transfered embryo	p value	Follicular fluid prolactin	p value
Group 1	Negative	2.3±0.7	0.016*	48.3±20.8	0.537
	Positive	3.1± 0.3		42.6±15.6	
Group 2	Negative	1.9 ±0.8	0.011*	30.0±10.3	0.546
	Positive	2.7± 0.9		27.9±10.5	

*: p<0.05 Mann Whitney U-test

as in long protocols. As a result this provides a shorter time for ovarian stimulation and lower doses of gonadotropins (12-14).

All participants in both groups had similar demographic data. Throughout the ART cycles, five separate samples were withdrawn as blood and FF. When biochemical assays were compared between the two groups, the antagonist group had significantly lower estradiol levels in hCG injection and oocyte aspiration period and lower levels of LH in hCG injection time. These results were related to the effects of antagonists and consistent with the study of Albano et al. (15) that had compared both GnRH analogs. In addition, pregnants had significantly higher levels of estradiol in both groups. We suggest that lower levels of estradiol in non pregnants were related to the low responses in the induction period.

We detected lower FF prolactin levels in the antagonist group than the agonist group in the period of hCG administration in contrast to Noyes et al. (16) data. We also evaluated the relationship between prolactin levels and pregnancy outcomes. Mendoza et al. (17) suggested that there was a significant relationship between higher FF prolactin levels and pregnancy in their study of FF fluid hormones and pregnancy outcomes. However our results revealed that, although it was not statistically significant, pregnant participants had lower levels of FF prolactin in both groups. When pregnants were compared within the two groups, the antagonist group had significantly lower levels than the agonists. These inconsistent results indicate that we need to perform more detailed researches.

There was prominent difference among aspirated oocytes between groups in favor of the agonist treatment and similar data were reported for the agonist group in long protocol by Ludwig et al. (18). It was suggested that the short induction period of the antagonist group causes these low numbers of oocytes and Wikland et al. (19) reported that increasing doses of gonadotropins in antagonist cycles may provide significantly higher numbers of oocytes. However, in our study, although both groups had similar stimulation periods and gonadotropin doses, the antagonist group had significantly lower numbers of oocyte. These results suggest that there may be other related factors about lower responses. In the antagonist treatments, lower levels of estradiol and number of oocytes reveal fewer ovarian hyperstimulation syndromes associated with a negative effect on pregnancy. It was proven that higher levels of estradiol have adverse effects on the luteal phase. Additionally, higher numbers of oocytes do not always mean that all these oocytes have a capacity of fertilization. Chen et al. (20) reported in their study that, although the number of oocytes in both groups were significantly different, they had similar fertilization rates, number of embryos, as in our study.

In the present study, leptin levels did not differ in the two groups. Noyes et al. (16) examined both serum and FF leptin levels at the beginning of induction and day of oocyte aspiration in agonist and antagonist groups. Results were similar for serum leptin levels in both groups, but FF leptin levels differ in favor of the antagonist group (15.3±1.4 versus 24±3.9 p=0.03).

Leptin has negative effects on various growth factors (IGF-1, TGF-beta) and hormones (insulin, glucocorticoids) that affect gonadotropin stimulated sex steroid hormones when utilized over 10 ng/ml (21-24). In addition, higher doses of leptin decrease ovarian estradiol synthesis and block the dominant follicle development and oocyte maturation. Serum and FF leptin levels in successful pregnancies were significantly lower than in women with failed conception (25-27). On the other hand, De Placido et al. (28) reported that FF leptin concentration of 20.25 ng/ml was the most reliable cut-off in predicting fertilization of oocytes. Hadrie at al. (21) reported that leptin values increased significantly from the first to the second trimester, decreased slightly in the third trimester and declined markedly 4-6 weeks after delivery. Abnormally low serum leptin levels were observed in women suffering spontaneous abortion in the first trimester of pregnancy (29). In our study, serum leptin levels were similar in both groups. Also we could not establish a relationship between serum leptin levels and pregnancy outcomes, similar to Gürbüz et al. (30) but contrary to Yang and Huang (26). In both groups, FF leptin levels were lower in pregnants than non pregnants, but this difference was not statistically significant. On the other hand, FF leptin levels were significantly higher than simultaneous serum leptin levels.

There is a controversy about the relationship between leptin and estradiol. Some authors suggest that high serum leptin levels might inhibit estradiol production through direct or indirect mechanisms (30-32). Others suggest that there was no correlation between these hormones (33, 34). We also could not find any correlation between leptin and both estradiol and prolactin. Moreover, we evaluated the same relationship between pregnancy outcomes and prolactin levels of FF. There is a controversy about the value of FF prolactin levels in predicting pregnancy. Some researchers suggested that PRL concentration of FF might be an additional parameter of oocyte maturation and fertilizability (35, 36). However, others suggested that higher levels of prolactin has adverse effects or no impact on pregnancy outcome (37, 38). In our research, pregnant women in both

groups had lower follicular fluid prolactin levels than non pregnants but this was not statistically significant. When pregnants were compared within the two groups, the antagonist group had significantly lower levels than agonists.

All researchers do agree on the impact of BMI on leptin levels (2, 39, 40, 41). We also observed a statistically significant correlation between leptin levels and BMI in group 2 in contrast to Group 1.

Successful outcomes of assisted reproductive technology rely on many factors such as the patients' age, number of transferred embryo, and quality of both embryo and implantation sites. It was suggested that levels of estradiol alterations might have influence over implantation through leptin. So, whether leptin has an impact on fertility or not should be clearly introduced. The leptin level alterations during ovulation induction which is different from natural cycles, may be related induction protocols.

As a result, both GnRH agonists and antagonists in short protocols had the same efficacy over pregnancy outcomes as suggested in literature. Although leptin is thought to be an important predictor of ovulation induction cycles, we could not introduce this relationship significantly both in serum and FF samples. There is a need for more randomized controlled researches in order to determine the physiopathologic mechanisms of leptin over ART.

Conflict of interest

No conflict of interest was declared by the authors.

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